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14. ABSTRACT
For this postdoctoral fellowship the specific role of selenoproteins in prostate carcinogenesis is being investigated using a cell culture model system. First, the biological consequence of varying allelic identities in the selenoprotein glutathione peroxidase (GPx-1) in response to selenium (Se) supplementation was determined. GPx-1 has either a leucine (leu) or proline (pro) amino acid at the 198 codon, and cancer risk has been shown to vary depending on the allelic identity. Our investigation showed that the prostate cell line LNCaP containing the amino acid leu at the 198 codon was more responsive to Se supplementation although its baseline GPx activity was lower compared to cells with a pro at the same codon. These studies have increased our understanding of the effect of genetic variations in GPx-1 on the response to dietary Se and would be relevant to the findings from the SELECT trial on the observed effects of Se supplementation. The second objective was to investigate the effect of reduced levels of selenoproteins on DNA damage and cell proliferation. Significant progress has been made in reducing GPx-1 levels in prostate cell lines using the siRNA technique. Efforts are now in progress to investigate the biological consequence of these reduced levels. It is being investigated whether reduced GPx-1 levels increase DNA damage caused due to UVC treatment and whether this effect is attenuated if Se is supplemented in the medium prior to UV treatment. Collectively, these studies will help determine whether selenium effects in prostate carcinogenesis are mediated through selenoproteins. Understanding the mechanism of action would help maximize benefits of Se as a chemopreventive agent.

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Selenoproteins, selenium, prostate cancer, glutathione peroxidase, DNA damage

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ANNUAL SUMMARY

INTRODUCTION

Epidemiological and experimental data over several years have provided new information indicating that selenium is an effective, non-toxic means to prevent several types of cancer, including cancer of the prostate. It is unknown whether the mechanisms of selenium action are mediated through selenium-containing proteins although genetic evidence supports a role for two selenoproteins, GPx-1 and Sep15 in this process. Loss of one of two copies of the GPx-1 gene is a common event in several cancers, and a polymorphic variant of GPx-1 was found to be associated with cancer risk. Similarly, allelic loss at the Sep15 locus occurs in several cancer types as well, and functional genetic variations in this selenoprotein gene may be involved with cancer risk as well. GPx-1 has anti-oxidant activity, while the function of Sep15 is unknown. It is hypothesized that GPx-1 and/or Sep15 levels are associated with prostate cancer risk and the increase in the activity of these proteins when humans are provided dietary selenium supplements help prevent that disease. This concept is particularly relevant to the SELECT trial, the largest prostate cancer prevention trial ever conducted, designed to investigate if selenium supplementation can prevent prostate cancer when provided to cancer-free men. This post-doctoral fellowship application proposed to investigate the role of these selenoproteins in prostate cancer using cell culture model systems.

RESEARCH ACCOMPLISHMENTS (According to tasks outlined in the statement of work)

1) Task I of this proposal aimed at exploring the biological consequence of Sep15 polymorphism in human prostate cell lines as a function of selenium availability. Specifically, the aim was to determine whether the naturally occurring polymorphic alleles of Sep15 produce differing amounts of the corresponding protein as a function of selenium availability in human prostate cells.

a) Screening cell lines for Sep15 and GPx-1 alleles.

To fulfill this aim, prostate cell lines were screened, in order to identify the Sep15 genotypes. In addition to identifying Sep15 alleles, the nucleotide identity at the polymorphic 198 codon of the GPx-1 gene of these same cells was also determined. The Sep15 gene was genotyped for the only reported polymorphisms at positions: CG/TA, CG/CG and TA/TA. The GPx-1 gene was genotyped to identify the nucleotide at the codon 198 polymorphism.

Nine independent prostate cell lines were screened in order to identify the genotypes for Sep15 and GPx- 1 selenoproteins. The identified genotypes are listed below in table 1:

Table 1: Selenoproteins Sep15 and GPx genotype in prostate cell lines.

Name of cell line	Genotype	
	Sep15	**GPX198**
1532NTPX	CG/TA	198P
1532CTPX	CG/TA	198P
1535NTPX	CG/CG	198P
1535CTPX	CG/CG	198P
1542NTPX	CG/TA	198P
1542CTPX	CG/TA	198P
LNCaP	CG/CG	198L
Du145	CG/CG	198L
PC3	CG/CG	198L

The following technical difficulties have been encountered in our investigation of the role of Sep15 in prostate carcinogenesis, due to which the proposed work on Sep15 in these prostate cell lines cannot be effectively pursued.

1) None of the screened cell lines displayed the TA/TA allele for the Sep15 gene, which we had proposed to investigate.
2) Good, stable antibodies to detect the expression of Sep15 are unavailable at this time, which has limited any further investigations on the function of this selenoprotein.

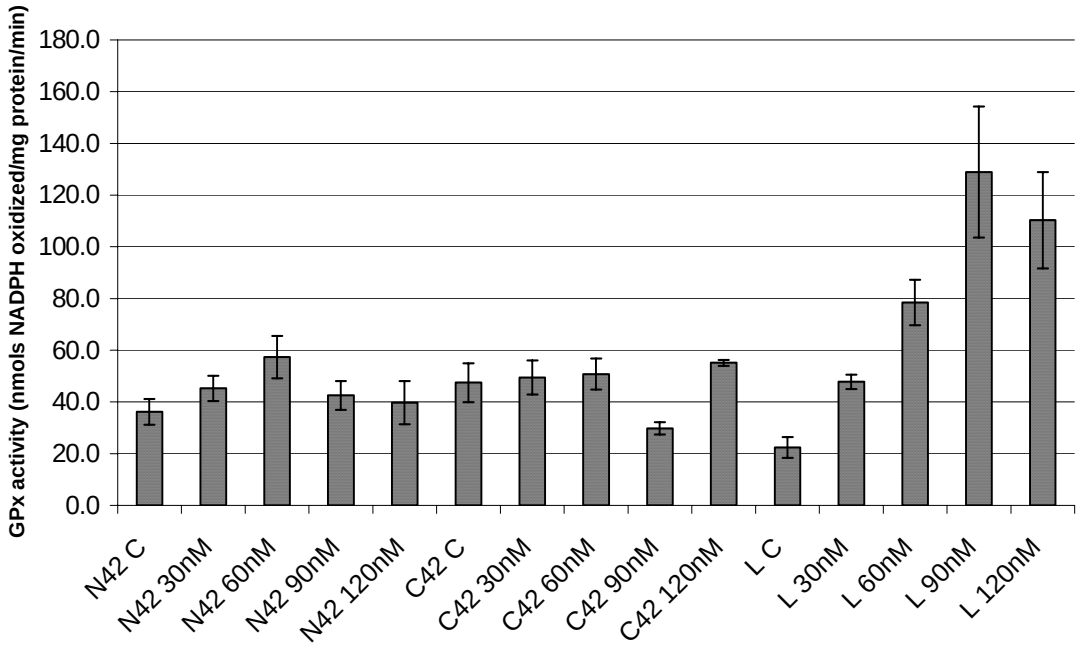
Future studies instead will use transgenic animals that express reduced Sep15 levels, along with reduced levels of other selenoproteins, to address this protein's role in prostate cancer risk and development.

b) Experiments with selenium supplementation of human prostate cell lines differing in GPx-1 gene allele identity.

Allelic identity of the GPx-1 gene was determined in prostate cancer cell lines. Among the screened cell lines, three (LNCaP, NPTX-42 and CPTX-42) were selected to investigate whether the presence of a leucine or proline containing allele affected GPx enzyme activity as a function of selenium availability. LNCaP and CPTX-42 are both prostate cancer cell lines, while NPTX-42 represents a normal cell line derived from normal tissue of the same individual from which the CPTX-42 line was derived. LNCaP cells contain the Leu allele, and both NPTX-42 and CPTX-42 cell lines contain the pro allele (Table 1)

GPx activity was assessed at 4 different concentrations of selenium (30, 60, 90 and 120nM) compared to untreated control cells. Response to 3 day selenium treatment in the cell lines is shown in Figure 1.

Figure 1: GPx activity in prostate cell lines with different allelic identity at 198 codon in response to selenium supplementation



N42 = NPTX42 Normal prostate cell line with Proline at GPx 198 codon
C42 = CPTX42 Prostate cancer cell line with Proline at GPx 198 codon
L = LNCaP Prostate cancer cell line with Leucine at GPx 198 codon
C = Control untreated cells
30nM = 30 nM Selenium supplementation
60nM = 60 nM Selenium supplementation
90nM = 90 nM Selenium supplementation
120nM = 120 nM Selenium supplementation

Key observations:

1) Baseline GPx activity was found to be lower in the LNCaP cells (Leu) compared to NPTX42 and CPTX42 cell lines (Pro). This difference was significant compared to CPTX42 cell line ($P = 0.04$). No difference in baseline GPx activity was observed between NPTX42 and LNCaP or NPTX42 and CPTX42 cells.
2) While both NPTX42 and CPTX42 cell lines did not show a significant increase in GPx enzyme activity following selenium supplementation at several doses, a significant induction in GPx activity was found in LNCaP supplemented with selenium at 30nM ($p < 0.001$), 90nM ($p < 0.05$) and 120 nM ($p< 0.05$) compared to untreated control cells.

These results indicate that although the baseline GPx activity was lower in the leu-containing LNCaP cells, the response to selenium supplementation in these cells was significant when compared to pro-containing NPTX42 and CPTX42 cells. The biological significance of this difference will be investigated.

2) Task II of this proposal aimed at assessing the consequence of reduced levels of GPx-1 and Sep15 on DNA damage levels in human prostate cells.

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a) Reduction of GPx-1 levels in human prostate cells using the siRNA technique.

In order to reduce GPx-1 levels using interfering RNA technology, 5 mRNA target sequences for the GPx-1 gene were identified using Ambion's siRNA target finder tool. The following table lists the sequences of the siRNAs.

Table 2: SiRNA target sequences for GPx-1 gene silencing

Target Sequence Number	Sequence	Position in gene sequence
TS 4	AAGGTACTACTTATCGAGAAT	192
TS 12	AATCTCCCTTGTTTGTGGTTA	536
TS 15	AAGAACGAAGAGATTCTGAAT	621
TS 16	AACGAAGAGATTCTGAATTCC	624
TS 17	AAGAGATTCTGAATTCCCTCA	628

- Using each target sequence, hairpin siRNA template oligonucleotides were synthesized for ligation into Ambion's pSilencer vector. As a negative control, the pSilencer vector without an inserted oligonucleotide was used.
- The hairpin siRNAs were cloned into the Ambion's psilencer 2.1-U6 hygromycin siRNA expression vector.
- Clones with siRNA inserts were identified by restriction enzyme digestion following amplification in a bacterial host.

Plasmids containing the target sequence (TS 15) or the control vector were transfected by electroporation into LNCaP cells using Amaxa biosystem's nucleofector. Transfected cells were selected with hygromycin and 12 colonies were picked for each plasmid.

b) Efficacy of GPx-1 siRNA gene silencing.

Each of the transfectants were expanded and screened for GPx enzyme activity in order to select a cell line with the reduced GPx activity as compared to control transfectants. Table 3 shows the GPx activities obtained using extracts prepared from these transfectants.

Table 3: GPx activity of siRNA LNCaP cell lines

Cell line	GPx activity (nmoles NADPH oxidized/mg protein/minute)
LNCaP control transfectants	23.7 ± 6.5
LNCaP GPx SiRNA transfectants	2.4 ± 0.580

c) Perform experiments to determine the effects of reduced selenoprotein levels on DNA damage and cell proliferation.

Work on this aspect of the proposal is in progress. In order to investigate the effects of reduced GPx levels on DNA damage, a UVC dose of 12 J/M^2 was selected for treatment. The following treatment groups were included for the control and GPx SiRNA transfectants:

1) Control cells (no treatment group)
2) Cells treated with 30nM sodium selenite for 5 days (Se only group)
3) Cells treated with a UVC dose of 12 J/M^2 at 5 days and harvested after 22 hours (UV only group)
4) Cells treated with 30nM sodium selenite for 5 days + UVC dose of 12 J/M^2 at 5 days and harvested after 22 hours (UV + Se group)

Harvested cells will be assessed for the following genes associated with stress and DNA damage responses in order to investigate the effects of reduced GPx levels in presence and absence of selenium supplementation.

1) Gadd45, a DNA damage inducible gene.
2) Total and phosphorylated Akt levels. Akt is a serine-threonine kinase that promotes cell survival by phosphorylating proteins involved in cell apoptosis and proliferation. Deregulation of the Akt pathway is a common event in many cancers and has evolved as a critical survival pathway in prostate cancer.

LIST OF REPORTABLE OUTCOMES

1) Manuscript: Paper submitted for review indicating that reduced selenoprotein levels accelerate prostate cancer development. (Title: Selenoprotein deficiency accelerates prostate carcinogenesis in a transgenic model).

2) Abstract: Abstract submitted to Experimental Biology 2006 conference for oral presentation (See Appendix)

CONCLUSIONS

My efforts supported by the awarded postdoctoral fellowship will help address the specific role of selenoproteins, including GPx-1 in prostate cancer. I have genotyped several human prostate cancer cell lines and established a relationship between GPx-1 genotype and the levels/induction of that protein. These studies increase our understanding of the effect of genetic variations in GPx-1 on the response of that protein to dietary selenium levels. I have reported significant progress towards the goal of reducing GPx-1 in cultured cells, which can now be used to study the biological consequences of reduced levels of that protein. In addition, we have established a unique animal model for the study of the role of selenium and selenium-containing proteins in prostate cancer risk. This model will be the focus of my efforts for the next funding period.

APPENDIX

Abstract submitted to Experimental Biology 2006 conference

Selenoprotein deficiency increases prostate carcinogenesis in a transgenic mouse model

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Considerable animal and human data point towards a chemopreventive role for selenium, however its mechanism of action remains unclear. Selenoproteins are likely involved in the anti-carcinogenic effects of selenium wherein genetic polymorphism data and antioxidant functions provide support for this role. In order to investigate the function of selenoproteins in prostate carcinogenesis in the absence of a variation in selenium intake, a transgenic mouse model was developed. These mice, referred to as i6A-/Tag expressed reduced levels of selenoproteins due to the presence of a mutant selenocysteine tRNA, and an increased risk for prostate cancer due to the directed expression of the SV40 large T and small t oncogenes in the prostate. The i6A-/Tag mice were compared to control WT/Tag for the presence, degree and progression of prostatic intraepithelial neoplasia (PIN). A clear difference in prostate histopathology was observed, with the selenoprotein deficient mice displaying an increased predisposition towards higher-grade lesions with time. In the early weeks significantly more prostates were normal in the WT/Tag compared to the i6A-/Tag mice, however as time elapsed a greater shift from low-grade to high-grade PIN was apparent in the selenoprotein deficient mice indicative of an accelerated development towards prostate cancer. These data implicate selenoprotein levels in prostate cancer progression.

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